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Applicant: Michael Meyrick Burrell and
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Serial No.: 991,451

Filed: December 16, 1992

Art Unit: 1804

For: MODIFICATION OF PLANT METABOLISM

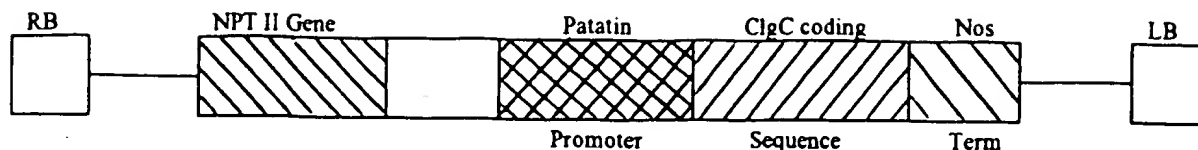
Examiner: D. Fox

DECLARATION UNDER RULE 37 CFR 1.132

I, Michael Meyrick Burrell, of 20 Histon Road, Cottenham, Cambridge, England, DO SOLEMNLY and SINCERELY DECLARE as follows:

1. I am the Michael Meyrick Burrell who is named as being an Applicant in respect of United States Patent Application Serial No. 991,451.
2. I am a scholar of Cambridge University and hold a PhD in Plant Biochemistry and a Degree in Natural Sciences with honours in Botany.
3. I initiated and supervised the conduct of the experimental procedures now to be related.
4. A chimaeric gene was constructed to express in potato tubers the *E. coli* enzyme ADPG pyrophosphorylase (EC 2.7.7.27). To achieve such construction the plasmid pFW4101 (NCIMB 40306) was employed, which plasmid contains a kanamycin resistance marker gene. The GUS coding region was removed from the pFW4101 plasmid and was replaced by the coding region from the *E. coli* gene *Glg C₁₆*. This resulted in new plasmid pFW4173, which is depicted below.





5. The plasmid pFW4173 was then electroporated into the strain C58#3 of *Agrobacterium tumefaciens* (NCIMB 40345). Leaf discs of the potato variety Prairie were cocultivated with these bacteria, following which regenerated shoots were obtained. Transformed shoots were selected by use of the kanamycin marker. Tubers were then derived from the transformed shoots and the tubers were analysed for ADPG pyrophosphorylase activity using a standard spectrophotometric assay method.
6. Tissue from the said tubers was also assayed in respect of sugar and starch. In this respect tissue was frozen in liquid nitrogen and was then powdered. The powder was extracted with hot ethanol. The ethanol soluble material was assayed to determine the sugar content, use being made for this purpose of a standard spectrophotometric assay method. The residual insoluble material from the extraction was assayed to determine the starch content, starch being measured as the amount of the insoluble material that could be converted to glucose by α amyloglucosidase and α amylose. The glucose was assayed using a standard spectrophotometric assay method.
7. The results of these assays are represented, for transformed Prairie lines designated (81, 87, 2) and (82, 85, 22), in the histogram herewith.
8. The histogram clearly shows that the transformation of lines 82, 85 and 22 resulted in enhanced ADPG pyrophosphorylase activity as compared with that of lines 81, 87 and 2 and that for lines 82, 85 and 22 this was associated with a decrease in sugar content and an increase in starch content of the potato tubers.
9. For the avoidance of doubt the term "ADPG pyrophosphorylase" as used above is an abbreviated form of the term "adenine diphosphoglucose pyrophosphorylase" as appearing in claims of U.S. S.N. 628,216.

I further declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that wilful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code, and that such wilful false statements may jeopardise the validity of the application or any patent issuing thereon.

Signature: Michael M. Lowell

Date: 5th March 1993